

NOTES

Conservation of a Symbiotic DNA Region in Soybean Root Nodule Bacteria

MATTHIAS HAHN AND HAUKE HENNECKE*

Mikrobiologisches Institut, Eidgenössische Technische Hochschule, ETH-Zentrum, CH-8092 Zurich, Switzerland

Received 6 April 1987/Accepted 22 June 1987

***Bradyrhizobium japonicum* USDA 311b110 contains a DNA region in which symbiotic genes and many repeated sequences are closely linked. Hybridization analysis revealed that this region was highly conserved in some *B. japonicum* strains (USDA 24, USDA 122, USDA 123, ATCC 10324, 61A24) but not in others (USDA 76, 61A76, 61A101). The genomic presence of multiple copies of one of the repeated sequences (RS α) appeared to be specifically characteristic for soybean root nodule bacteria, including the fast-growing *Rhizobium fredii*, which carries most of these RS α copies on the symbiotic plasmid.**

Definition of the bacterial species *Bradyrhizobium japonicum* is based on its symbiotic relationship to soybean (*Glycine max* (L.) Merr.) and on its slow growth rate (8). *B. japonicum* was shown to comprise strains which fell into at least two divergent homology groups (6, 7, 14). Investigation of the organization of symbiotic genes has been focused on strain USDA 110, a member of homology group Ia according to Hollis et al. (6). In this strain, two clusters of symbiotic genes have been characterized (4, 5, 10). One of them (cluster I), containing the nitrogenase structural genes *nifDK* and *nifH* and several other *nif* and *fix* genes, was found to be located in an unusual genetic environment in which spontaneous deletions can occur and which contains an accumulation of repeated sequences (4, 9). Using hybridization experiments, we wished to determine whether a similar organization of symbiotic genes and repeated sequences also exists in other *B. japonicum* strains. The *B. japonicum* strains that were included in the analysis are listed in Table 1. Total DNA from these strains was isolated (3) and digested with restriction enzymes, and genomic blots were hybridized at 60°C to different probes.

Hybridization of plasmid pRJ7001 (which carries a 809-base-pair *Hind*III fragment of *nifH*; 2) to *Eco*RI-digested DNAs allowed us to divide the strains into two groups; one group, consisting of strains USDA 24, USDA 122, USDA 123, ATCC 10324, and 61A24, showed the same 2.8-kilobase (kb) hybridization band as strain 110*spc*4, whereas the other group, consisting of strains USDA 76, 61A76, and 61A101, hybridized with a band of 10.5 kb (Fig. 1, and data not shown). Three strains of the first group, namely, USDA 24, USDA 122, and USDA 123, showed an identical pattern of additional, more weakly hybridizing bands (Fig. 1). These bands were due neither to partial digests nor to vector hybridization, since the same DNA samples showed only one hybridizing band with a *nifD* probe (Fig. 2B), and the bands also appeared when a purified *nifH* fragment was used as a probe. Hence, reiteration of *nifH* sequences, which has been found in several diazotrophic bacteria (e.g., reference

11), does apparently also occur in certain *B. japonicum* strains.

In strain USDA 110, one copy each of two different repeated sequences, RS α and RS β , and the 5'-terminal part of *nifD* are located on a 5.6-kb *Eco*RI fragment (9). To look for the presence of a similar fragment in the other strains, their DNA was hybridized sequentially to probes containing RS α DNA, *nifD* DNA (5'-terminal part), and RS β DNA (Fig. 2). With one exception, all strains of group I showed an *Eco*RI band of 5.6 kb hybridizing to all three probes; in strain USDA 123, instead, a 5.1-kb band was found (lanes 5 in Fig. 2). In contrast, the strains corresponding to homology group

TABLE 1. *Bradyrhizobium* and *Rhizobium* strains used in this work

Strain	Symbiotic phenotype ^a	Source or reference
<i>B. japonicum</i> USDA 110 <i>spc</i> 4	Nod ⁺ Fix ⁺	(12)
<i>B. japonicum</i> USDA 24	Nod ⁺ Fix ⁻	NifTAL ^b (17)
<i>B. japonicum</i> USDA 122	Nod ⁺ Fix ⁺	USDA ^c
<i>B. japonicum</i> USDA 123	Nod ⁺ Fix ⁺	Agrigenetics ^d
<i>B. japonicum</i> ATCC 10324	Nod ⁺ Fix ⁺ (type strain)	ATCC ^e
<i>B. japonicum</i> 61A24	Nod ⁺ Fix ⁻	Nitragin ^f (16)
<i>B. japonicum</i> USDA 76	Nod ⁺ Fix ⁺	USDA ^c
<i>B. japonicum</i> 61A76	Nod ⁺ Fix ⁺	NifTAL ^b
<i>B. japonicum</i> 61A101	Nod ⁺ Fix ⁺	NifTAL ^b
" <i>Bradyrhizobium</i> " sp. (<i>Lupinus</i>) ATCC 10319	Nod ⁻ (type strain) ^g	ATCC ^e
" <i>Bradyrhizobium</i> " sp. (<i>Crotolaria</i>) 32H1	Not tested	Nitragin ^f
<i>R. fredii</i> USDA191 <i>str</i> 1	Nod ⁺ Fix ⁺	Agrigenetics ^d
<i>R. fredii</i> USDA191C3	Nod ⁻	Agrigenetics ^d
<i>Rhizobium</i> sp. (<i>Lablab</i>) NGR234	Nod ⁻ (bumps only)	(15)

^a On *Glycine max* (L.) Merr. cv. Williams 82.

^b NifTAL Project, University of Hawaii, Paia.

^c U. S. Department of Agriculture, Beltsville, Md.

^d Agrigenetics Advanced Science Co., Madison, Wis.

^e American Type Culture Collection, Rockville, Md.

^f Nitragin Company, Milwaukee, Wis.

^g Nod⁺ Fix⁺ on *Lupinus polyphyllus*.

* Corresponding author.

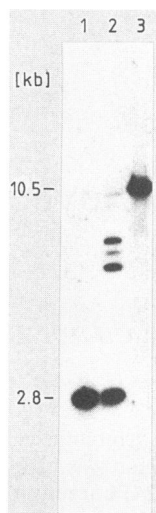


FIG. 1. Autoradiograph showing *nifH*-specific hybridization in different *B. japonicum* strains. Total DNA from strains USDA 110*spc4* (lane 1), USDA 24 (lane 2), and USDA 76 (lane 3) was digested with *EcoRI*, separated by agarose gel electrophoresis, blotted to a nitrocellulose filter, and hybridized with radioactive plasmid pRJ7001 carrying an 809-base-pair *HindIII* fragment of the *B. japonicum* 110 *nifH* gene.

II did not show a single fragment hybridizing to all three probes. Thus, the region upstream of *nifD* has been highly conserved in the group I strains. Consistent with the results of Stanley et al. (14), strains of groups I and II strongly diverge in their pattern of hybridizing fragments. Nevertheless, all *B. japonicum* strains showed multiple hybridization bands with RS α and RS β .

When hybridized to RS α DNA, the group I strains showed common *EcoRI* bands in addition to that of 5.6 kb, indicating a conservation of other RS α -containing fragments as well (Fig. 2A, arrowheads). To confirm this assumption, and perhaps to correlate the conserved fragments with those

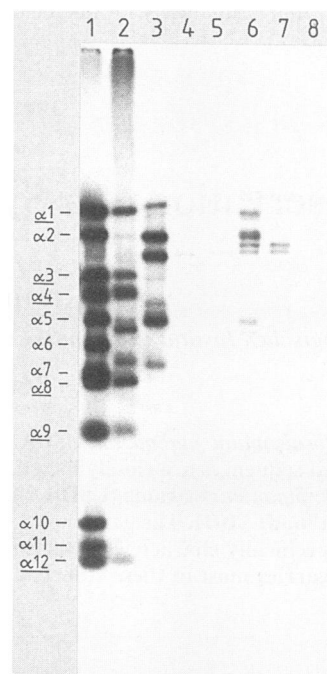


FIG. 3. Occurrence of RS α sequences in different *Bradyrhizobium* and *Rhizobium* strains. An RS α -specific DNA probe was hybridized to a blot containing *XhoI*-digested total DNA of the following strains: *B. japonicum* USDA 110*spc4* (lane 1), ATCC 10324 (lane 2), and USDA 76 (lane 3); "*Bradyrhizobium*" sp. (*Lupinus*) ATCC 10319 (lane 4); "*Bradyrhizobium*" sp. (*Crotolaria*) 32H1 (lane 5); *R. fredii* USDA 191*str1* (lane 6) and USDA 191C3 (lane 7); *Rhizobium* sp. (*Lablab*) NGR234 (lane 8). The left margin shows the numbering of the RS α fragments in strain 110*spc4*; the underlined copies are those which are present around cluster I region and which are conserved in several group I strains such as ATCC 10324 (lane 2).

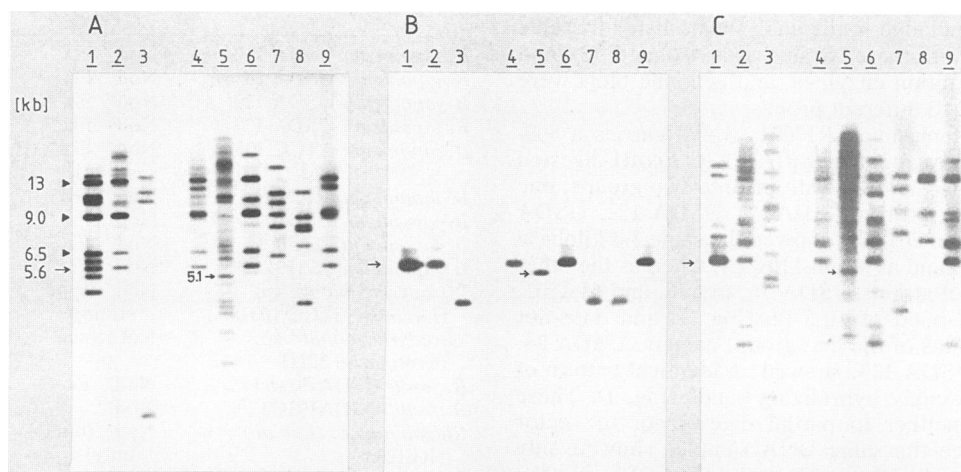


FIG. 2. Conservation of a 5.6-kb *EcoRI* fragment in different *B. japonicum* strains. The three autoradiographs shown were obtained after successive hybridization of the same nitrocellulose filter to RS α DNA (A), *nifD* DNA (B), and RS β DNA (C). The fragments hybridizing to all three probes are indicated by arrows. Other fragments which were conserved in several strains are marked by arrowheads. The lanes contained *EcoRI*-digested total DNA from the following *B. japonicum* strains: USDA 110*spc4* (lane 1); USDA 24 (lane 2); USDA 76 (lane 3); USDA 122 (lane 4); USDA 123 (lane 5); 61A24 (lane 6); 61A76 (lane 7); 61A101 (lane 8); and ATCC 10324 (lane 9). Certain lane numbers are underlined; these lanes contain DNA from homology group I strains.

known in strain USDA 110, *Xho*I-digested total DNAs of group I strains were again hybridized to RS α DNA. With the exception of strain USDA 123, all strains had six hybridization bands in common; they corresponded exactly to those RS α copies in strain USDA 110 which had been located in the large, unstable region surrounding cluster I, namely, RS α 1, - α 3, - α 4, - α 8, - α 9, and - α 12 (Fig. 3, lanes 1 and 2, and data not shown; 4, 9).

Taken together, these results indicate that, in *B. japonicum* group I strains, a very similar organization may exist not only of the *nif* and *fix* genes in cluster I but also of the surrounding region which contains an accumulation of repeated sequences. This region appears to be particularly well conserved, since (i) *Xho*I fragments with RS α copies which are located outside of the unstable region in strain USDA 110 were not found in other strains (Fig. 3, lanes 1 and 2), and (ii) the restriction fragment pattern of total DNAs from different group I strains, when visualized by ethidium bromide staining after agarose gel electrophoresis, showed only partial or no similarities (not shown).

In view of the high conservation of RS α within *B. japonicum* strains, we were interested in investigating the possible occurrence of similar sequences in other *Rhizobium* or *Bradyrhizobium* strains. The strains analyzed in the hybridization experiment shown in Fig. 3 are listed in Table 1. Only weak RS α -hybridizing bands were observed in the DNAs of "*Bradyrhizobium*" sp. (*Lupinus*) ATCC 10319, and no significant hybridization was seen in the DNA of "*Bradyrhizobium*" sp. strain 32H1 (Fig. 3, lanes 4 and 5). This result was somewhat unexpected for strain ATCC 10319, which had been reported to be very closely related to group I strains of *B. japonicum* (6, 7). Upon hybridization with the *nifD* and *nifH* probes described before, the signals obtained with strain ATCC 10319 were much weaker than those obtained with any of the *B. japonicum* strains (not shown). Examination of its symbiotic properties showed that strain ATCC 10319 was Nod⁺ Fix⁺ on *Lupinus polyphyllus* but Nod⁻ on *Glycine max*. From these results one might speculate that strain ATCC 10319 has a nonsymbiotic genetic background, similar to that of *B. japonicum* strains, while being strongly divergent with respect to the organization of genes for symbiosis.

Several bands hybridizing to RS α were shown in the DNA of *Rhizobium fredii* USDA 191str1 (Fig. 3, lane 6), a fast-growing strain which effectively nodulates American soybean cultivars (1, 13). The significance of these bands was corroborated by the hybridization pattern of the symbiotic (Sym) plasmid-free derivative USDA 191C3, which lacked several bands present in the wild-type strain (Fig. 3, lanes 6 and 7). This demonstrates that the majority of the hybridizing fragments are located on the Sym plasmid. In contrast, several other *Rhizobium* strains, including *Rhizobium* sp. strain NGR234 (Fig. 3, lane 8), *Rhizobium* sp. strain ORS571, *R. trifolii*, *R. leguminosarum*, and *R. phaseoli* (not shown) did not hybridize to RS α . It thus appears as if RS α occurs exclusively in soybean root nodule bacteria.

We thank the organizations listed in Table 1 for sending bacterial cultures. S. Hitz is gratefully acknowledged for expert technical assistance.

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